**A cell-based model of the dynamics of in vitro micro-aggregation**

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Micro-aggregation of precursor cells enhances cell performance upon differentiation both in vitro and in vivo [1]. Although the underlying mechanisms have remained largely unknown, it has been demonstrated that micro-aggregation creates a three-dimensional environment that recapitulates aspects of embryonic differentiation via auto- and paracrine signaling [2]. Moreover, micro-aggregation allows cells to establish a favorable mechanical micro-environment, by remodeling their cytoskeleton, depositing ECM and strengthening adhesion. Patterning micro-wells into a substrate is an ideal platform to generate micro-aggregates in a controlled and high throughput manner. The use of a non-adhesive substrate - e.g. agarose – prevents the cells from attachment and hence results in spherical micro-aggregates.

In general, the formation of cell aggregates is characterized by two phases. First, the cells actively migrate towards each other to form a loose cluster of spherical cells. Next, the cell cluster condensates and contracts into a smooth spherical micro-aggregate. The dynamics of micro-aggregate formation differ depending on cell type, dimensions of the micro-well and number of cells per well.

In this study, we developed a model which describes the mechanism of micro-aggregation by focusing on the behavior and mechanics of individual cells (See Figure 1). The model includes two important biological phenomena. Firstly, the cells perform a directed random walk which is influenced by chemo-taxis. Secondly, the adhesion energy for a given contact between two cells increases over a specified time. The cells' positions are updated by solving the equation of motion which is formulated for an over-damped system.

The simulations are compared to time lapsed microscopy images of in vitro micro-well cell aggregation of ATDC5 cells. The model predicts that the same cell behavior that results in realistic single cell migration also gives rise to the observed collective cell migration of the complete aggregate. Furthermore, a parameter study shows that spherical aggregates will be created as long as cell migration forces are high enough to overcome the initial forces of adhesion, and the directed component of cell migration is high enough to prevent the formation of multiple aggregates.

References

[1] Moreira Teixeira, L. S., et al. High throughput generated micro-aggregates of chondrocytes stimulate cartilage formation in vitro and in vivo. European cells & materials 23 (2012): 387-399.

[2] Dahlmann, Julia, et al. The use of agarose microwells for scalable embryoid body formation and cardiac differentiation of human and murine pluripotent stem cells. Biomaterials 34.10 (2013): 2463-2471.

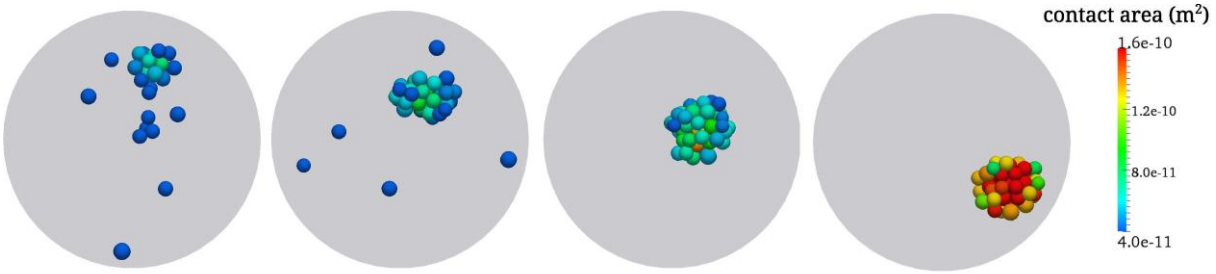


**Figure 1**: Simulated micro-well cell aggregation. From left to right: 160s, 224s, 384s and 1140s.